Jensen (1989) conducted feeding experiments utilizing *Elysia viridis* (Montagu) by transferring slugs found on *Codium fragile* to the cellular alga *Chaetomorpha linum* (Miller) Kutzing, and those found on *Chaetomorpha* to the coencytic *Codium*. In general, the slugs experienced great difficulty in changing their feeding habits, with time lags of up to two weeks. These time delays were attributed to learning periods but may have reflected in part the time necessary to restructure the radular apparatus. Unfortunately, the respective radulae were not examined and compared. However, as there are three regional populations of *Elysia viridis* in Europe, each feeding primarily on a different genus—*Codium, Bryopsis,* and *Chaetomorpha* (Jensen, 1989)—any real difference in radular morphology could be verified prior to prolonged feeding experiments. In passing, it is worth noting that Jensen's *Codium*-derived *E. viridis* were smaller (*n* = 17; average length 1.45 cm) than those collected off *Chaetomorpha* (*n* = 30; average length 2.13 cm).

I suggest that the survival strategy of *Placida dendritica* is to prey upon a variety of algae, including reliable perennials and desirable transients. If the veligers settle upon *Codium* they must contend with defensive chemicals and structures, and the result is a relatively small body size, rapid production of slender teeth, and fewer eggs laid. If the veligers find *Bryopsis* instead, they grow rapidly, produce fewer, more massive teeth, reach body lengths twice that of the *Codium*-limited individuals, and produce far more eggs.

Such a biochemical interplay between alga and ascoglossan, involving defenses, feeding effort, and nutrient value could explain the startling size discrepancies that one encounters sporadically when sampling populations over extended time periods. For example, the European ascoglossan *Limapontia depressa* Alder & Hancock, 1862, is usually only 2–3 mm in length, but in August 1969, in the Isefjord, Denmark, the late Henning Lemche (*in litt.*) found a swarm of hundreds of spawning animals most of which were an exceptional 8 mm in length, and their egg masses contained 800–1000 eggs. In contrast, collections of specimens of the usual 2 mm size, gathered on the same day but at a different locality, had egg masses containing only 155 eggs. Similarly, in the Minas Basin, Nova Scotia, the ascoglossan *Alderia modesta* (Loven, 1844) can grow to 15 mm, nearly twice its "normal" size, but this happens at irregular intervals, often years apart. *Elysia chlorotica* is usually considered "large" at 20–30 mm, but on occasion, in Minas Basin tidal marshes, entire populations can reach 35–45 mm. Gibson *et al.* (1986) reported an exceptional phenomenon concerning this species. Much of the local population in the summer of 1983 and 1984 was without chloroplasts. Apparently, viable chloroplasts from *Vaucheria* were unavailable, yet the slugs appeared healthy and were actively spawning. Most of the non-green *Elysia* were 6–12 mm in length and only the few green individuals attained larger body sizes.

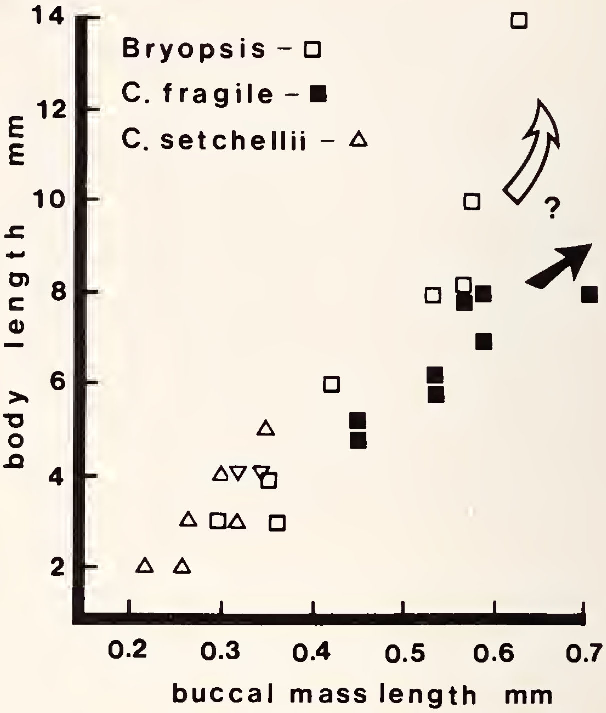

Figure 6

Relationship of body length and length of buccal mass to type of alga fed upon by *Placida dendritica* from Oregon.

CONCLUSIONS

The above observations, considered with the ecological studies of Clark & DeFreese (1987), emphasize how entrained and responsive are the life histories and anatomies of ascoglossans to their algal prey. It is also evident that these responses are open to experimental verification and manipulation at laboratories where controlled culturing techniques are available for both larvae and algae. The standard method of testing of adult ascoglossans using algal choice situations to establish a food preference spectrum for a particular slug species could be misleading. The "preferred" food of adults may simply depend upon the alga that veligers or larvae first encountered and to which they effectively adapt morphologically and physiologically during their brief life history period.

ACKNOWLEDGMENTS

The author is indebted to Ms. Cynthia D. Trowbridge, Oregon State University, for providing the algal-specific samples of *Placida dendritica* that provided the impetus to re-examine information from other localities.

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THE VELIGER

Trail Following in *Littorina irrorata*: The Influence of Visual Stimuli and the Possible Role of Tracking in Orientation[1]

by

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Abstract. The marsh periwinkle *Littorina irrorata* can detect and follow mucous trails previously deposited by conspecifics. Preliminary observations revealed that the tendency of *L. irrorata* to track may be influenced by the presence of visual directional stimuli. When tested in a field arena, both the frequency and degree of trail following exhibited by periwinkles were significantly higher under conditions in which the marker's trail was deposited in response to a visual stimulus. No significant differences were found between controls and treatments in which a visual orientational cue was absent for marker snails. These data suggest that mucous trails deposited by visually oriented marker snails differ in informational content from trails laid by non-oriented snails. Microscopic and elemental analysis of trails deposited by the same snail while crawling in the absence and presence of a directional stimulus revealed thin threadlike structures possibly involved in determining trail polarity, but no noticeable differences were observed in the overall physical or chemical structure of the two groups of trails.

INTRODUCTION

The influence of mucous trails on the movements and orientation of gastropods has received considerable attention, but most studies have focused on the role of trail-following behavior in limpet homing. The marsh periwinkle *Littorina irrorata* (Say, 1822), like many marine prosobranchs, frequently follows conspecific mucous trails in the same direction in which they were originally deposited (HALL, 1973). Although recent evidence suggests that trail following in this species increases locomotor efficiency by reducing the force required to crawl across the marsh substratum (TANKERSLEY, 1989), the role of intraspecific trails and trail polarity in determining the movements and orientation of *L. irrorata* in the marsh has yet to be demonstrated.

Littorina irrorata typically is active at low tide among stands of the cord grass *Spartina alterniflora* (BINGHAM, 1972; HAMILTON, 1978a) which the snails ascend when inundated by the advancing tide (HAMILTON, 1976). Although numerous shore-living gastropods have been shown to utilize a variety of orientational mechanisms and directional stimuli including light, gravity, water currents, and waves to determine their movements and maintain their patterns of distribution on the shore (UNDERWOOD, 1979), the zonal orientation and short-term movements of *L. irrorata* on the marsh substratum are largely mediated by local visual cues, especially grass stalks (HAMILTON, 1977a, 1978b; HAMILTON & WINTER, 1982). When displaced from its natural habitat onto bare sand, *L. irrorata* crawls toward areas of vegetation (HAMILTON, 1978b). Avoiding areas devoid of *Spartina* is particularly adaptive since snails locate and climb grass stalks and in so doing may avoid predators at high tide (HAMILTON, 1976; WARREN, 1985) and reduce heat stress (MCBRIDE *et al.,* 1989). Because directional information present in *L. irrorata* trails lasts for at least 60 min following deposition (STIRLING & HAMILTON, 1986), trails deposited by conspecifics may provide information necessary for the relocation of plant stems during tidal inundation or serve as a supplement to other orientational guideposts, including visual cues.

Numerous studies have revealed that animals often possess redundant orientational mechanisms which facilitate the performance of the same tasks under different environmental conditions (ABLE, 1980). Because most previous examinations of trail following and orientation in *Littorina irrorata* have either employed visual stimuli (STIRLING &

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Hamilton, 1986; Tankersley, 1989) or controlled for the potential influence of old trails on test surfaces (Hamilton, 1977a, 1978b; Hamilton & Winter, 1982), it has been impossible to ascertain the role of conspecific trails in determining the orientation behavior of *L. irrorata* or explore any relationship that might exist between mucous trails and other directional stimuli, especially visual landmarks.

Examinations of the trail-following behavior of *Littorina irrorata* under controlled conditions revealed that when marker (trail-layer) snails were deprived of visual directional stimuli, the tendency of tracking *L. irrorata* to follow the marker's trail was substantially reduced. This observation suggested that trails of visually oriented snails might contain important trail-specific information, in addition to directional (polarity) information, that may not be present or detectable in the trails of non-oriented snails foraging or crawling randomly on the marsh substratum. The present study addresses the hypothesis that trails produced by visually orienting marker snails differ from those of non-visually orienting snails with respect to their potential to elicit trail following by tracker snails.

MATERIALS AND METHODS

General Procedures

Mature *Littorina irrorata* (shell length 17–23 mm) were obtained from marshes adjacent to the Florida State University Marine Laboratory, Turkey Point, Franklin County, Florida. Snails not used immediately after collection (see microscopic examination of trails) were maintained in plastic aquaria partially filled with natural seawater (32–34‰ salinity) under fluorescent light on a 12L:12D photoperiod. All snails were used within five days of collection.

Arena Experiments

Trail following by *Littorina irrorata,* in response to trails that had been deposited in the presence or absence of a visual directional stimulus, was assessed using an experimental arena resembling that of Hamilton (1978b) (Figure 1; see Tankersley, 1989, for a detailed description). The arena consisted of two 40-cm-diameter circular wooden platforms constructed of 1.9 cm thick plywood. A 30-cm-diameter glass plate was inserted into the center of the upper platform and served as the test surface. A 10 cm high white plastic collar was placed on top of the upper platform to limit the influence of external visual stimuli. The arena was then mounted on top of a PVC pipe and placed 1.3 m above the salt marsh substratum among stalks of *Spartina.*

Twenty pairs of marker and tracker *Littorina irrorata* were tested under four treatment combinations in which a visual stimulus (12 cm × 3 cm black vertical bar) either was attached at a random location on the inside of the collar (Present treatments) or removed (Absent treatments) (Table 1). For each replicate, a marker snail was selected from the nearby salt marsh and placed in the center of the arena with its aperture facing a randomly chosen direction and allowed to crawl until it reached the outer edge of the test surface. The snail was then removed and the arena and collar were rotated 90°. A tracker snail was then placed next to the beginning of the marker's trail and allowed to crawl until it reached the collar. Once the tracker reached the edge of the arena, the trails of both snails were outlined and traced onto paper. Between trials the test surface was cleaned with detergent and 90% ethanol (Hamilton, 1977b).

The length of the marker's trail (L_m), the tracker's trail (L_t), and the portion of the tracker's trail coincident with the marker's trail (L_c) were measured using a curvimeter (Figure 2). A tracker's trail had to overlap the marker's trail completely in order to be considered coincident. The degree of trail following exhibited by each tracking snail was estimated using the following Coincidence Index (C.I.) adopted from Townsend (1974):

$$\text{C.I.} = L_c/(L_m \cdot L_t)^{1/2}$$

Snails that either stopped or failed to reach the edge of the arena within 5 min were excluded from the analysis. In order to estimate the degree of coincidence expected by chance, pairs of randomly selected marker trails were superimposed to serve as control values for L_m, L_t, L_c, and C.I.

The lengths of marker, tracker, and coincident trails were compared using a one-way analysis of variance (ANOVA). Coincidence Indices for treatment and control groups were analyzed via a Kruskal-Wallis one-way layout test and distribution-free multiple comparisons based on mean ranks (Hollander & Wolfe, 1973).

Microscopic Examination of Trails

Microscopic comparisons of trails deposited by the same snail while crawling toward an artificial stalk (black vertical bar) and in the absence of this directional stimulus were conducted using both light (Nikon Optiphot compound scope; differential interference contrast [Nomarski]) ($n = 15$) and scanning electron microscopy (SEM) (Jeol 840-II scanning electron microscope) ($n = 10$). Because most fixation procedures destroyed trails or appeared to alter their natural structure, mucous trails deposited on glass microscope slides (40 mm × 25 mm) were either air dried (light microscopy) or frozen in liquid nitrogen (SEM) immediately after deposition. Following freezing, slides containing trail samples for SEM were critical-point dried, mounted on specimen stubs, coated with gold-palladium, and examined at 20 kV.

The elemental composition of trails of visually oriented and non-oriented marker snails ($n = 7$) were compared using energy dispersive microanalysis. Trail samples were obtained by allowing snails to crawl over carbon SEM stubs (14-mm diameter) in the presence or absence of a

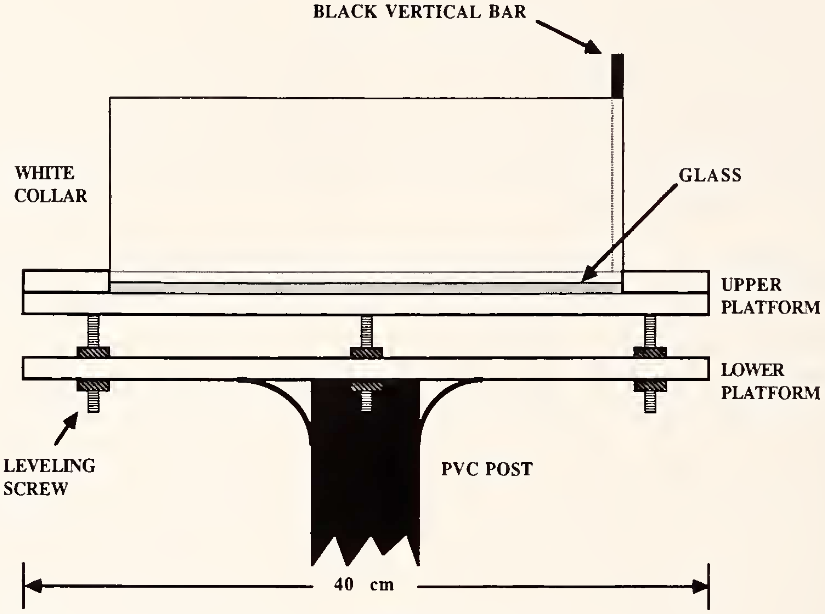

Figure 1

Side view of experimental arena.

visual directional stimulus. Trails and stubs were dried in a desiccator for 1 h and coated with carbon for observation in a Cambridge S4-10 stereoscan scanning electron microscope equipped with a Tracor Northern TN 2000 microanalyzer.

RESULTS

When placed in the arena, tracker *Littorina irrorata* detected and followed conspecifics' trails in 65% of all trials ($n = 80$). The frequency of trail following varied from 85% ($n = 20$) in trials in which the visual orientational stimulus was present for both snails to 35% ($n = 20$) in trials in which the vertical bar was present only for tracker snails. The trails of marking snails crawling in the absence of the visual stimulus (A/P or A/A) were significantly longer and more circuitous than those of snails crawling toward the vertical bar, but not significantly longer than controls ($F = 8.04$, d.f. = 4, 95; $P < 0.001$; Figure 3).

Table 1

Experimental treatments used to determine the effect of a visual orientational cue on the degree of trail following.

Treatment	Artificial stalk	
	Marker	Tracker
P/P	Present	Present
P/A	Present	Absent
A/P	Absent	Present
A/A	Absent	Absent
Control		

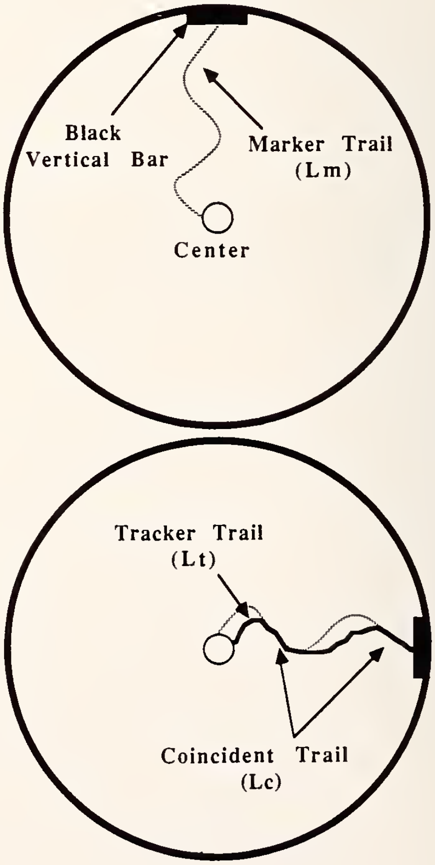

Figure 2

Typical trails deposited by marker and tracker snails crawling in either the presence or absence of a visual directional stimulus.

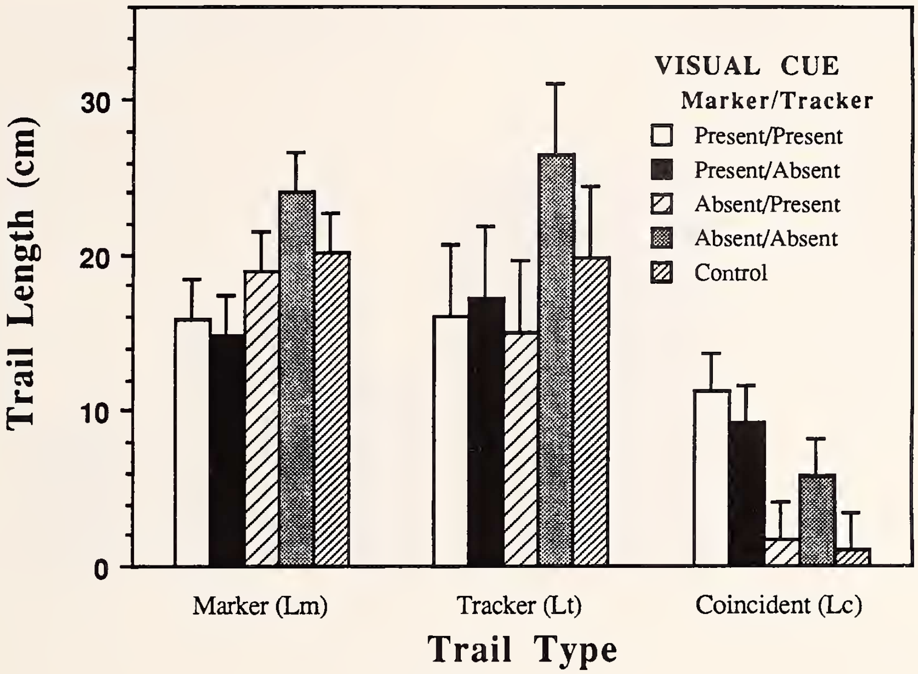

Figure 3

Mean marker, tracker, and coincident trail lengths and 95% comparison intervals for the four experimental treatments in which a visual directional stimulus was either present or absent for marker and tracker *Littorina irrorata*. Pairs of marker trails were randomly selected and superimposed to serve as controls.

The lengths of tracker trails for the Absent/Absent (A/A) treatment were significantly longer than the tracker trails for all other treatments ($F = 15.46$, d.f. = 4, 95; $P < 0.001$; Figure 3). When coincident trails were standardized by the length of the marker and tracker trails (calculated C.I.), the degree of trail following was significantly greater under conditions in which the marker trail was laid in the presence of a visual directional stimulus ($H = 43.79$, d.f. = 4; $P < 0.001$; Figure 4). No significant differences were found between the two treatments in which the artificial stalk was present for marker snails (P/P and P/A). Similarly, the treatments in which the orientational cue was absent for marker snails (A/P and A/A) were not significantly different from each other or from the controls.

Microscopic examination of trails using either Nomarski optics or SEM revealed no noticeable differences in the overall physical and morphological structure of trails laid down by the same snail while crawling in the absence or the presence of a visual directional stimulus. Both trails were thin mucous mats of parallel threadlike structures oriented along the trail's axis (Figure 5). The strands are similar to those previously described by STIRLING & HAMILTON (1986) for stained *Littorina irrorata* trails and resemble those reported for other gastropods including *Helix aspersa* (SIMKISS & WILBUR, 1977) and the tracking mud snail *Ilyanassa obsoleta* (BRETZ & DIMOCK, 1983). A comparison of the elemental composition of air-dried marker trails using X-ray microanalysis revealed significant concentrations of sulfur, sodium, magnesium, potassium, chlorine, and calcium, regardless of whether they were laid down in the presence or absence of a visual stimulus. Although the relative concentrations of the elements differed slightly between samples, the overall composition of the trails and the seawater control were not significantly different.

DISCUSSION

Although trail following is an obvious component of the behavior of *Littorina irrorata*, few published studies have attempted to establish the natural conditions under which it occurs or determine its functional role. This study has demonstrated that *L. irrorata* pursues conspecific mucous trails that have been deposited in response to visual orientational cues more frequently and with greater precision (coincidence) than trails that were laid in the absence of such cues. The frequencies of trail detection and tracking not only were higher in treatments with a directional cue present for marking snails, but snails also tended to follow greater proportions of markers' trails over longer distances (Figures 3, 4). Furthermore, the presence of a directional stimulus for tracker snails (treatments A/P and P/P) did not significantly influence their responses to a conspecific's trail. These results suggest that mucous trails deposited by visually orienting marker snails contain different or more discernible information than trails deposited by snails

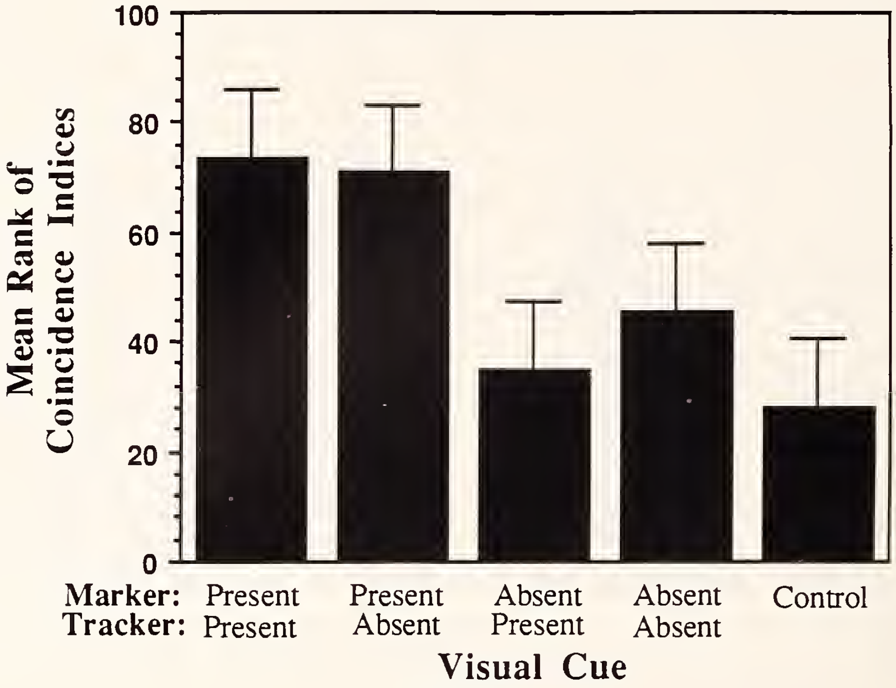

Figure 4

Mean ranks and 95% comparison intervals (Kruskal-Wallis one-way layout test) for the calculated coincident indices (C.I.).

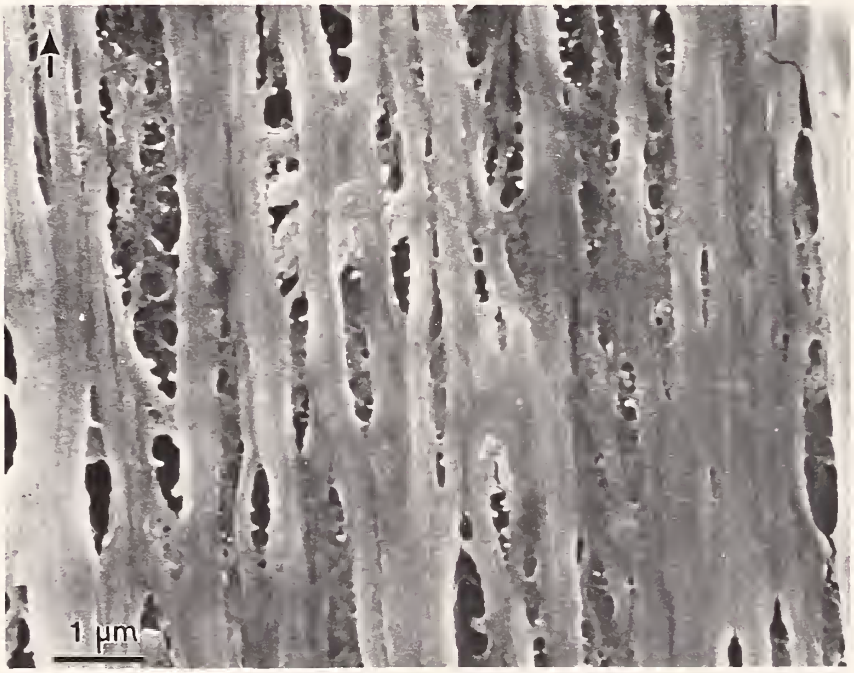

Figure 5

Scanning electron micrograph of a mucous trail deposited by *Littorina irrorata*. Arrow indicates the direction of trail deposition.

crawling in the absence of a visual directional cue. Therefore, trail following might serve as an important orientational mechanism facilitating the location of *Spartina* stalks by *Littorina* as the tide advances.

The role of mucous trails in molluscan orientation has been well documented, especially for homing gastropods. Although several proposed homing mechanisms have been extensively studied, including external cues, kinesthetic, and topographic memory (FUNKE, 1968), direct olfaction and contact chemoreception with previously deposited trails usually are considered most plausible (COOK *et al.,* 1969; COOK, 1969, 1971; COOK & COOK, 1975; COOK, 1979a, b, 1980; MCFARLANE, 1980; ROLLO & WELLINGTON, 1981). COOK (1979a) proposed that homing is accomplished via a dual chemosensory mechanism in which separate pheromones are responsible for trail following and distance chemoreception. Observations of the homing behavior of several terrestrial snails and slugs support this hypothesis (ROLLO & WELLINGTON, 1981). The use of chemical trails for homing is not restricted to gastropods and has been reported for other mollusks including the chitons *Acanthozostera gemmata* (THORNE, 1968) and *Acanthopleura gemmata* (CHELAZZI *et al.,* 1987).

The heightened response of trackers to trails deposited by marker snails that had been oriented to visual cues suggests that those mucous trails possess encoded information that triggers or enhances tracking behavior. Although the mechanisms that mediate either tracking and homing behavior or the detection of trail polarity have not been determined for any gastropod, most workers hypothesize that either chemical pheromones or physical discontinuities in trails are involved (COOK *et al.,* 1969; COOK, 1971; TROTT & DIMOCK, 1978; COOK, 1979a, b; BOUSFIELD *et al.,* 1981; BRETZ & DIMOCK, 1983). Thus, one might hypothesize that structural or chemical differences exist between the trails of visually oriented versus non-oriented *Littorina irrorata,* making those trails distinguishable to a tracking snail.

Molluscan mucus, although mostly water (91–98%) (DENNY, 1983, 1984), is chemically complex and frequently contains high molecular weight glycoproteins, proteins, amino acids, carbohydrates (predominantly mucopolysaccharides) and lipids (HUNT, 1967; WILSON, 1968; TRENCH *et al.,* 1972; TRENCH, 1973; WHITEHEAD, 1978; GRENON & WALKER, 1980; DENNY, 1983). BOUSFIELD *et al.* (1981) propose that metabolic substances, including known molluscan attractants such as butyrate and propionate, might be responsible for the triggering and specificity of tracking behavior by *Biomphalaria.* Informational differences in mucous trails of the homing pulmonate *Onchidium verruculatum,* which deposits inbound trails that contain little or no directional information relative to outbound trails, also have been reported (MCFARLANE, 1981). MCFARLANE (1981) hypothesized that the reduction in information in the trail of *O. verruculatum* is caused by a decrease in the deposition of a specific trail substance in inbound versus outbound trails. More recently CHELAZZI *et al.* (1987) suggested that the homing chiton *Acanthopleura gemmata* minimizes the chances of following a conspecific's trail by depositing a trail containing both species-specific and quasi-individual information. Regulation of the information content of mucous trails might be accomplished by independent neuronal control of chemicals secreted from different mucous cells present in the snail's pedal gland (CHASE & BOULANGER, 1978).

A less likely explanation for the tendency of tracker snails to follow trails deposited by visually orienting markers is that non-orienting markers may deposit some substance in their pedal mucus that deters trail following. When displaced from its natural habitat and disoriented, *Littorina irrorata* might secrete a chemical with its mucus that inhibits the tracking response or otherwise renders the trails unattractive to conspecifics. It is unlikely that the trail is made repellent, per se, because tracker snails readily crossed trails made by non-oriented markers, and in fact would follow short segments thereof. However, the marine opisthobranch *Navanax inermis* has been shown to secrete a bright yellow alarm pheromone into its pedal mucus when mechanically disturbed by an investigator or attacked by a potential predator (SLEEPER *et al.,* 1980). A tracking *Navanax* quickly stops trail following and deviates from such a trail. *Littorina irrorata* does exhibit an avoidance response to secondary metabolites present in some marine macroalgae (TARGETT & MCCONNELL, 1982). Displaced and disoriented snails might deposit similar metabolites or substances with their mucus.

Microscopic comparison of trails laid in the presence versus the absence of a visual stimulus revealed no obvious physical, morphological, or chemical differences between the two types of trails. Although trails were composed of thin mats of threadlike strands resembling those that may be involved in determining trail polarity in *Ilyanassa obsoleta* (BRETZ & DIMOCK, 1983), neither type of trail possessed any unique morphological structures or chemical elements that were not found reciprocally. Because the microanalysis technique that was employed is limited to detection of inorganic elements only, the organic components of the mucous trails have not been examined.

As with many other species, *Littorina irrorata* most likely possesses a suite of orientational abilities integrating several sensory stimuli and orientational mechanisms, including visual, tactile, and chemical cues (ABLE, 1980). Complex orientational repertoires have been described for other shore-living animals, especially arthropods (HERRNKIND, 1972). The influence of environmental stimuli, including visual objects, on the orientation of *L. irrorata* in the marsh has been studied extensively (BINGHAM, 1972; HAMILTON, 1978b; HAMILTON & WINTER, 1982). Previously deposited mucous trails, as well as other non-visual mechanisms such as geotaxis, might augment visual information or serve as substitute reference systems when snails are deprived of visual and other stimuli, or when cues are rendered ambiguous (*e.g.,* when submerged or in darkness). Moreover, the location of vegetative areas may